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A Clinical Study on the Removal of Gingival Melanin Pigmentation with the CO_2 Laser

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Background and Objective: **In a previous study, the possibility of removal of dog gingival melanin pigmentation with CO_2 laser therapy was reported. The present study was designed to investigate the effect of the CO_2 laser on human gingival pigmentation and evaluate the clinical outcome.**
Study Design/Materials and Methods: **A CO_2 laser (output: 6–8 W, pulse duration: 0.2 seconds) was irradiated on the melanin pigmented gingival surface of 10 patients, aged 20–49 years. Follow-up clinical and histopathological evaluations were performed.**
Results: **The CO_2 laser was effective in removing melanin pigmentation in all patients. In the histopathological study, no pigmented-laden cells nor any inflammatory cell infiltration was observed following laser irradiation. No re-pigmentation was seen in any case in the first year. However, four of seven cases showed re-pigmentation at 24 months. The re-pigmentation was almost equal to the preoperative state.**
Conclusions: **The CO_2 laser has proved to be another effective, safe, and easily applicable therapy for the removal of gingival melanin pigmentation. Lasers Surg Med 25:140–147, 1999.**

Key words: clinical evaluation; de-pigmentation; re-pigmentation; laser therapy; melanin pigmentation

INTRODUCTION

Specific targeting of melanosomes may allow for laser therapy of pigmented cutaneous lesion in dermatology and plastic surgery [1–3]. In dentistry, melanin pigmentation of the gingiva is considered to be an esthetic disorder rather than a disease [4]. Treatment of such cases usually involves traditional, chemical, and cryosurgery [4–6]. Surgery with gingivectomy normally requiring local anesthesia, incision, and postoperative management with a surgical pack is necessary [7]. On the other hand, chemical surgery with phenol may have a toxic side effect [8]. Therefore, many people are hoping for a simple alternative technique instead of these surgical techniques in the treatment of this disorder.

Nd:YAG and Argon lasers are reported to be useful in removing gingival pigmentation due to their deep tissue penetrability and selective destruction of the pigmented cells found in the basal cell layer under the epithelium [9,10]. Color dependency and tissue penetrability are thought to be necessary for the treatment of melanin pigmentation. However, it has been reported that depths of thermal damage of Argon and Nd:YAG lasers are extend up to 200 μm and 600 μm, respectively [11]. Therefore, such penetrability may damage the underlying alveolar bone covered by thinner oral mucosa.

The CO_2 laser is another popular and available device in the clinical field. It has not been used to remove melanin as it was thought to lack

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TABLE 1. Patients Status*

Case no.	Age	Sex	Color density	Smoker
1	24	M	Moderate	Yes
2	49	F	Moderate	Yes
3	20	M	Moderate	Yes
4	20	F	Mild	No
5	45	F	Moderate	No
6	24	M	Severe	Yes
7	24	F	Moderate	Yes
8	24	M	Moderate	Yes
9	25	M	Moderate	Yes
10	35	M	Moderate	No

*Mild: Pink to slightly brown. Moderate: Deep brown or black. Severe: Mixed in color.

deep tissue penetrability and color dependency [12]. However, the depths of thermal damage of CO_2 laser have been reported to extend from 50 to 100 μm [11], which might cause less damage to the oral mucosa. CO_2 laser irradiation is almost completely absorbed by cellular water within the superficial irradiated surface [12]. Therefore, if CO_2 laser therapy is proved to be effective in the removal of melanin with less damage to the surrounding tissue, it could provide an alternative technique.

In a previous study, we reported that the removal of gingival melanin pigmentation with the CO_2 laser was possible in an animal experiment [13]. In this study, we examined the effect of CO_2 laser irradiation on human gingival melanin pigmentation and evaluated the clinical outcome.

MATERIALS AND METHODS

Subjects

A total of 10 patients with bilateral melanin pigmentation (extensive) at the anterior portion of the upper and lower gingiva including six males (20–35 years old) and four females (20–49 years old) attending the outpatient clinic at Showa University Dental Hospital were selected (Table 1).

All patients were considered healthy and were not using any specific medicine for this study.

Smoking Habits

At baseline, seven of 10 patients (70%) were recorded as current smokers (Table 1). The patients gave us full or verbal consents after precise explanation of the purpose of this trial. Furthermore, patients were told that they were completely free to withdraw from our study at any time should they feel any discrepancy.

Fig. 1. Laser device: An opelaser-03R (Yoshida Dental Mfg. Co., Ltd., Tokyo, Japan) was used.

Laser Device

Figure 1 shows the laser device used in this study. An opelaser-03R manufactured by Yoshida Dental Mfg. Co., Ltd. (Tokyo, Japan) was used. The 10.6 μm wavelength laser beam was delivered through an articulated arm system (operation modes: continuous wave). This laser device contains a lens in the handpiece for converging the beam within a 0.2 mm area, which is used as a laser-scalpel. However, to avoid surplus damage to the gingival tissue, this lens was removed.

The Therapeutical Procedure

Prior to irradiation, the gingival color was classified into three groups according to a modified Dummet et al. criteria [14]: mild (pink to slightly brown), moderate (deep brown or black), and severe (mixed in color) as shown in Table 1. The laser was irradiated in a similar manner to our previous study [13]. The pulse duration was 0.2 second with a defocused spot of 4 mm in diameter. In slight to moderate cases, the irradiation output was fixed at 6 W (9.6 J/cm^2). In a severe case (Case No. 6), the irradiation output was 8 W (12 J/cm^2). Figures 2–7 shows the therapeutical procedure. Figure 2 shows the preoperative condition. Surface anesthesia (Xylocaine® Pump Spray, Astra, Sweden) with cotton pellets was applied to the surface of the gingiva (Fig. 3), whereas local anesthesia was applied only in case of pain. The CO_2 laser was irradiated at first on one side of the pigmented gingiva guided by the laser handpiece. The laser was kept approximately 8 mm from the gingiva and a defocused beam with non-contact was used (Fig. 4). The tissue was irradiated until the lased area showed a

Fig. 2. Preoperative appearance showed moderate and extensive melanin pigmentation.

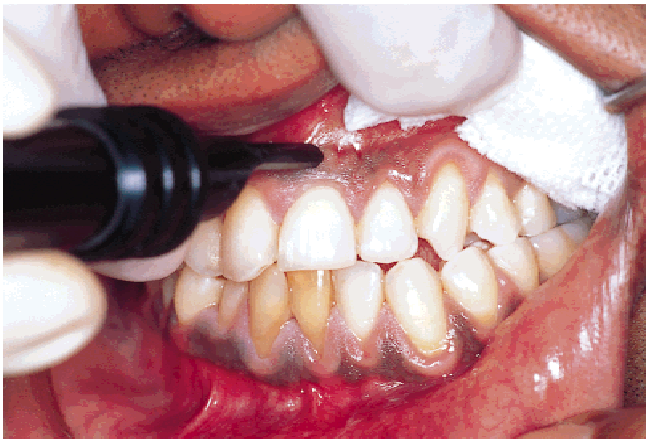

Fig. 4. The laser was kept approximately 8 mm from the gingiva and a defocused beam with non-contact was used.

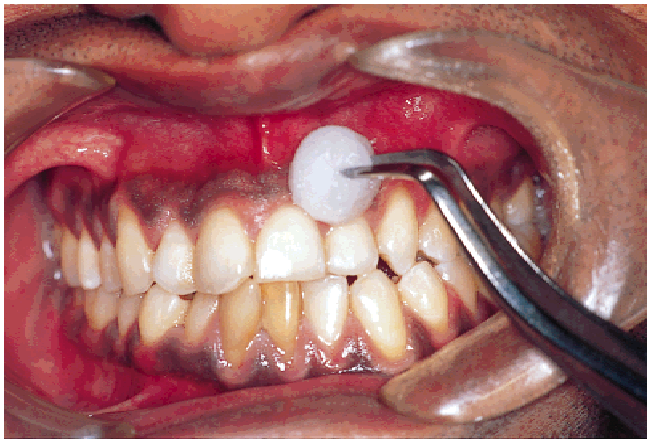

Fig. 3. Surface anesthesia with cotton pellets was applied to the gingival surface.

Fig. 5. The lased area showed a change of opaque white or bulla-like appearance.

change of opaque white or bulla-like appearance and any carbonization was avoided (Fig. 5). The irradiation time was related to the severity and extension of the pigmentation. In mild to moderate cases, usually one pulse in each spot was required to achieve opaque white or bulla-like appearance, while two or three pulses were required in the severe case. After one week, re-irradiation was performed on the remaining area (Fig. 6). The treatment was then administered on a weekly basis until a normal pink color of the gingiva was achieved (Fig. 7). A total of three to five treatments were required for the above purpose. Subsequently, the other side was treated with the same method.

Clinical and Histological Evaluation

The effect of the CO_2 laser was evaluated at various intervals during follow-up therapy: immediately after irradiation, one week following the first irradiation, at one month, 12 months (nine cases), and 24 months (seven cases) following completion of the therapy. Histological examination was performed in three cases before and at one week following irradiation. Biopsies were taken from the unlased and lased areas of the gingiva. Samples were then fixed with buffered formalin solution and embedded in paraffin. Histological sections (4 μm) were examined using hematoxylin-eosin staining and Masson staining.

RESULTS

During Laser Irradiation

Almost all patients complained of a slight burning sensation during laser irradiation under surface anesthesia. Local anesthesia was applied in two cases: one was due to the patient's fear

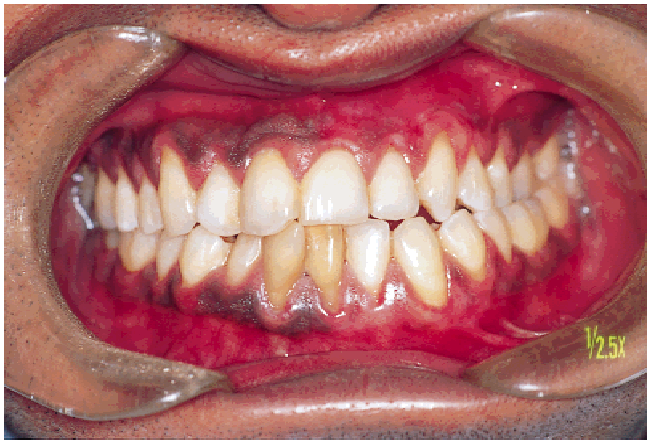

Fig. 6. One week after irradiation, the gingival surface showed a significant reduction in melanin pigmentation on the left side. However, melanin pigmentation still remained in the papillary region. Re-irradiation was performed at the remaining area.

Fig. 7. The gingiva showed de-pigmentation on the left side following re-irradiation and the completion of the therapy. Subsequently, the right side was treated by the same method.

during laser irradiation (Case No. 5) and the other was due to application of a strong output of 8 W in the case of severe pigmentation (Case No. 6).

Immediately After Irradiation

The lased gingival surface showed a change to an opaque white and bulla-like appearance (Fig. 5). No hemorrhage nor any epidermal detachment was observed. The damage-lased gingiva was slight in comparison with the conventional surgical method.

1 Week Following Irradiation

In all cases, the gingival surface showed a significant reduction in melanin pigmentation compared with the unlased gingiva. However, some remaining pigmentation was observed, especially in the region of the papilla (Fig. 6). The gingiva showed de-pigmentation on that side following re-irradiation (Fig. 7).

TABLE 2. Clinical Results*

Case no.	1 M (n = 10)	12 M (n = 9)	24 M (n = 7)
1	A	A	B
2	A	A	B
3	A	A	Un
4	A	A	A
5	A	A	A
6	A	Un	B
7	A	A	Un
8	A	A	Un
9	A	A	B
10	A	A	A

*A, neither inflammation nor hemorrhage nor re-pigmentation; B, re-pigmentation; Un, unknown.

Clinical Observation

The results of clinical observation at 1 month, 12 months, and 24 months following completion of the therapy are shown in Table 2. Clinical photographs are presented in Figures 8–10 at the preoperative, 12 months, and 24 months following completion of the therapy. Figures 11–14 show the histological appearance before and at one week.

All patients showed satisfactory results throughout the entire examination period. The gingiva showed a normal clinical appearance, pink in color (Fig. 9, 10). In addition, no inflammation nor hemorrhage was found in 9 of 10 patients. In only one case (Case No. 6), slight inflammation was found following laser irradiation (Fig. 16).

Histological Examination

Before irradiation, the pigmentation consisted of melanin granules in the basal cell layer with hematoxylin-eosin staining (Fig. 11). The melanin granules were stained with dark brown in Masson staining (Fig. 12). After irradiation, no inflammatory cell nor any tissue damage was observed with hematoxylin-eosin staining (Fig. 13). Moreover, melanin granules were not observed with Masson staining (Fig. 14).

Re-Pigmentation

No re-pigmentation of the gingiva was seen in any case during the first year. However, four of seven patients returned to almost the preopera-

Fig. 8. Preoperative appearance showed extensive pigmentation of moderate density.

Fig. 9. The gingiva showed a normal clinical appearance, pink in color, 12 months following completion of the therapy.

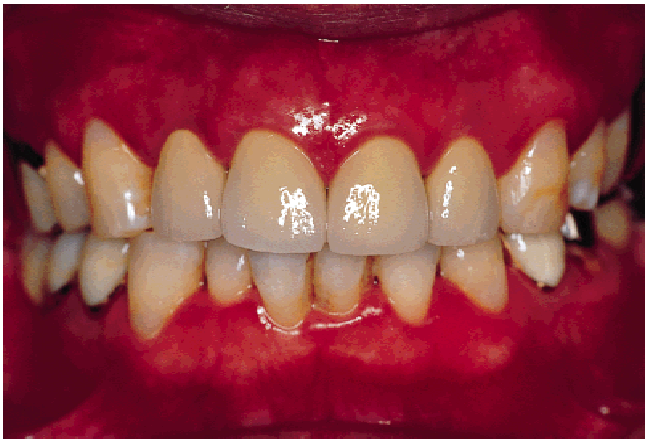

Fig. 10. Twenty-four months following the completion of therapy. The gingiva maintained a normal clinical appearance with no incidence of re-pigmentation. In addition, to improve the patient's esthetic value, a temporary crown was replaced with a porcelain crown on maxillary bilateral incisor.

Fig. 11. In hematoxylin-eosin staining, the pigmentation consisted of melanin granules in the basal cell layer (arrow), before irradiation. Original magnification ×33.

tive state of pigmentation at 24 months (Fig.15–17). There was some de-pigmentation found scattered in only the irradiated area.

DISCUSSION

In a previous study, we reported the removal of gingival melanin pigmentation with satisfactory results by CO_2 laser irradiation in dogs [13]. In this present study, we clinically applied irradiation to remove melanin pigmentation on human beings and to evaluate the clinical outcome.

The Therapeutic Result

In this study, the CO_2 laser proved to be effective for the removal of melanin pigmentation. All patients showed a normal gingival appearance following treatment and except in one case, no inflammatory reactions nor any morphological abnormalities were recognized throughout the entire observation period. Slight inflammation occurred in one severe case of pigmentation and due to use of a strong output of 8 W (Fig. 16). Difficulties were incurred when removing melanin in the region of the gingival papilla. This may be due to the high activity of pigmented cells in this area. Re-irradiation was usually necessary in the papillary region and was continued until a normal pink color appearance was achieved. Moreover, in the postoperative clinical course, none of the patients developed hemorrhage nor any infection except for a momentary sensation of heat at the irradiated area. Although therapeutic effects varied

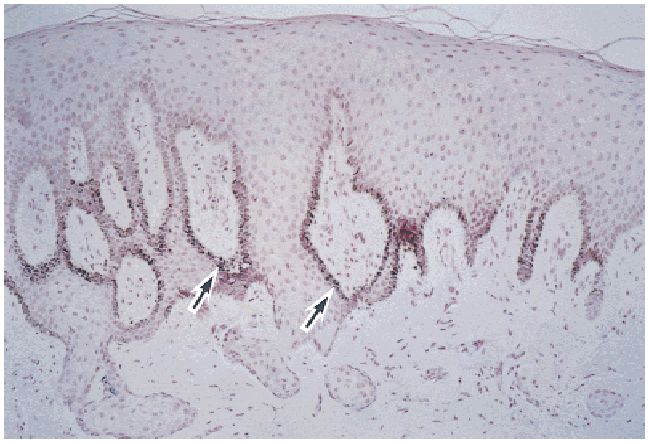

Fig. 12. In Masson staining, melanin granules were stained with dark brown (arrow) before irradiation. Original magnification ×33.

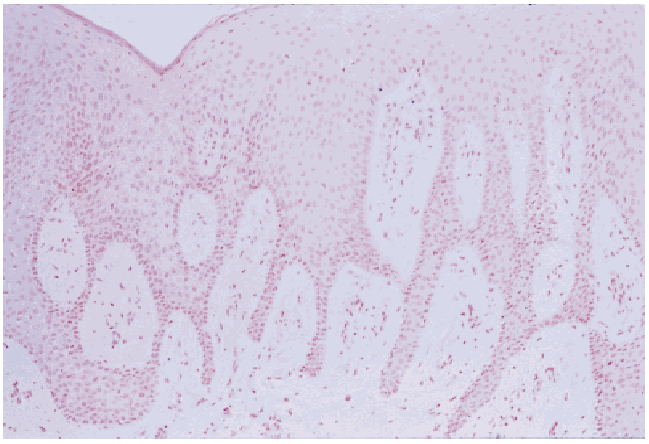

Fig. 14. Melanin granules were not observed with Masson staining after irradiation. Original magnification ×33.

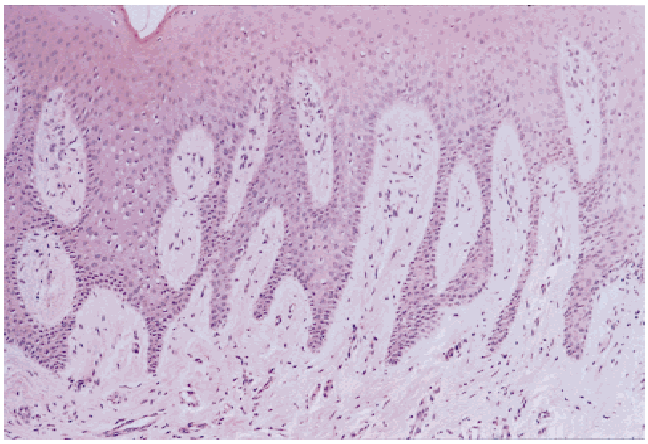

Fig. 13. No inflammatory cell nor any tissue damage was observed with hematoxylin-eosin staining after irradiation. Original magnification ×33.

Fig. 15. Preoperative appearance showed extensive pigmentation of severe density.

among the patients, repeated irradiation produced favorable results in all those who still had pigmentation after the first irradiation. Upon histological examination, melanin-laden cells were found to have been eliminated from the underlying gingiva.

Comparison with Therapeutic Technique

Previous studies have reported several therapeutic techniques for removal of gingival melanin pigmentation, such as traditional (e.g., gingivectomy), chemical (e.g., phenol), and cryosurgery [4–6]. However, laser therapy has the following advantages: (1) it causes less hemorrhage and infection, (2) therapeutic duration is shorter and technically easier, (3) minimal side effects. Moreover, when laser irradiation is applied in a non-contact manner, there is a low risk of infection. Therefore, laser irradiation could be considered superior to conventional surgical techniques.

Nd:YAG and Argon lasers are reported to be useful for removing gingival melanin pigmentation under local anesthesia [9,10]. However, the oral mucosa is thinner than the skin tissue. As the depths of thermal damage of the CO_2 laser extend up to 50 μm and the depths of Argon and Nd:YAG lasers are extend from 200 μm to 600 μm, respectively [11,12], it is thought that the CO_2 laser technique would be more desirable because it does not damage the deep tissue. Moreover, there is no report of carcinogenesis due to laser absorption by DNA. As the wave length of the CO_2 laser is 10.6 μm, far from the absorption maximum of deoxytibonucleic acid (266 nm), the possibility of carcinogenic effects can be excluded [15]. Therefore, CO_2 laser therapy can be consid-

Fig. 16. Following completion of the therapy, the gingival surface showed a normal clinical appearance. However, slight inflammation occurred due to use of a strong output of 8 W.

Fig. 17. Re-pigmentation at 24 months. The re-pigmentation was almost equal to the preoperative condition. There was some de-pigmentation found scattered in only the irradiated area.

ered as another safe alternative procedure to the high-powered lasers for the removal of melanin pigmentation because of less damage to the oral tissue from reaching depth.

Mechanism

Melanin is formed by dendritic melanocytes in the basal and spinous layers of the gingival epithelium. It is synthesized in organelles within the cells called premelanosomes or melanosomes [16]. CO_2 laser therapy must reach the basal cell layer to effectively treat melanocytes. The CO_2 laser is characterized by its surface tissue absorption and a lack of melanin selectively [12,17]. However, we considered the following: since the keratin layer of the gingiva is far thinner than that of the skin, the CO_2 laser most likely reaches the basal cell layer and degenerates the pigmented cells containing melanosomes using heat which may cause absorption. However, the mechanism underlying the removal of gingival melanin pigmentation in the present study has not yet been clarified, further research should be done before drawing any conclusions from these observations.

Re-Pigmentation

In the present study, re-pigmentation was only observed in four of seven patients at 24 months. In gingivectomy, the same results of re-pigmentation were reported by Dummet et al. and Perlmutter et al. [18,19]. Dummet et al. found re-pigmentation in six of nine patients within 49–120 days due to migration of the pigmented cells from surrounding areas. In a separate study, Perlmutter et al. examined two patients treated with gingivectomy and found de-pigmentation in both patients over the first two years and one case returned to the preoperative condition at seven years. Therefore, it has been found that CO_2 laser therapy showed comparable results with previous studies of gingivectomy. Surgery with gingivectomy normally requiring local anesthesia, incision and postoperative management with a surgical pack is necessary [7]. Therefore, CO_2 laser therapy seems comfortable to patients. However, the mechanism of re-pigmentation is not yet clarified. Perlmutter et al. reported that migration of active melanocytes could be the possible mechanism [19]. Although these studies did not mention any relationship between melanin pigmentation and smoking, the cases showing melanin pigmentation may have been smokers. Araki et al. reported that melanin pigmentation was frequently observed in smokers and that the manifestation rate among smokers was no longer different between Asians and Europeans [20]. In our present study, re-pigmentation was observed in four smokers at 24 months. As only seven patients were available for a long term study, it is premature to conclude that the habit of smoking is related to the incidence of re-pigmentation. Further research is needed to determine if any relationship exists between re-pigmentation and smoking habits.

In conclusion, the CO_2 laser has proved to be another effective, safe, and easily applicable therapy for the treatment of gingival melanin pigmentation because of less damage to the oral mucosa from reaching depth.

REFERENCES

1. Tong AKF, Tan OT, Boll J, Parrish JA, Murphy GF. Ultrastructure: effects of melanin pigment on target specificity using a pulsed dye laser (577 nm). J Invest Dermatol 1987;88:747–752.
2. Anderson RR, Margolis RJ, Watanabe S, Flotte T, Hruza GJ, Dover JS. Selective photothermolysis of cutaneous pigmentation by Q-switched Nd:YAG laser pulses at 1064, 532 and 355nm. J Invest Dermatol 1989;93:28–32.
3. Oshiro T. Laser treatment for Naevi. In: Laser treatment for Naevi. England: West Sussex, 1995;121–232.
4. Glickman I, Smulow JB. Gingiva Pigmentation. In: Periodontal disease. Philadelphia: WB Saunders Co., 1974. p 4.
5. Hirschfeld I, Hirschfeld L. Oral pigmentation and a method of removing it. Oral Surg Oral Med Oral Pathol 1951;4:1012–1016.
6. Tal H, Landsberg J, Kozlovsky A. Cryosurgical depigmentation of the gingiva—a case report. J Clin Periodontol 1987;14:614–617.
7. Kinoshita S, Wen R. A color atlas of periodontics. St. Louis, Tokyo: Ishiyaku EuroAmerica, Inc., 1985;190–196.
8. Ayress S. Dermal changes following application of chemical cauterants to aging skin. Arch Dermatol 1960;82:578–585.
9. Matsumoto K, Ochi K, Tachibana H, Wakabayashi H. Study on the removal of the melanin pigmentation by Nd:YAG laser. Jap J Conserv Dent (in Japanese)1986;29: 1543–1547.
10. Trelles MA, Verkruysse W, Segui JM, Udaeta, A. Treatment of melanotic spots in the gingiva by Argon laser. J Oral Maxillofac Surg 1993;51:759–761.
11. Absten GT, Joffe SN. Laser-tissue interactions. In: Lasers in medicine. 2nd edition. London: Chapman and Hall Ltd., 1989. p 16–21.
12. Lanzafame RJ, Hinshaw JR. Laser physics. In: Color atlas of CO_2 laser surgical techniques. St. Louis, Tokyo: Ishiyaku EuroAmerica, Inc., 1988. p 3–12.
13. Nakamura Y, Funato A, Wakabayashi H, Matsumoto K. A study on the removal of the melanin pigmentation of dog gingiva by CO_2 laser irradiation. J Clin Laser Med Surg 1992;10:37–42.
14. Dummett CO, Barens G, Sakumura JS. Attitudes towards normal pigmentations of the oral tissues. Quintessence Int 1981;10:1115–1122.
15. Green HA, Margolis R, Boll J. Unscheduled DNA synthesis in human skin after in-vitro ultraviolet-excimer laser ablation. J Invest Dermatol 1987;89:201–204.
16. Carranza FA. The tissues of the periodontium. In: Glickman's clinical periodontology. 5th edition. Philadelphia: WB Sanders Co., 1979. p 3–32.
17. Boulnois JL. Photophysical processes in recent medical laser developments, a review. Lasers Med Sci 1986;1:47–66.
18. Dummett CO, Ala T, Bolden TE. Postsurgical clinical repigmentation of the gingivae. Oral Surg Oral Med Oral Pathol 1963;16:353–365.
19. Perlmutter S, Tal H. Repigmentation of the gingiva following surgical injury. J Periodontol 1986;57:48–50.
20. Araki S, Murata K, Ushio K, Sakai R. Dose-response relationship between tobacco consumption and melanin pigmentation in the attached gingiva. Archives of Environmental Health 1983;38:375–378.